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Electrostatic Control of Protein-Surface Interactions

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14. ABSTRACT
The long-term vision of this research is to develop, characterize, and exploit surface chemistries that create stable protein-surface interactions by mimicking biological interfaces. We are pursuing this goal by chemically functionalizing well-defined and well-characterized surfaces with structured peptides. These peptides, which can be synthesized with any residual functional group, generate an electrostatic surface that will interact with proteins introduced from solution with chemically- and structurally-encoded specificity. In the current grant period, we have

15. SUBJECT TERMS
protein-surface interactions, protein electrostatics, surface chemistry, surface characterization

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Lauren Webb
UU	UU	UU	UU		19b. TELEPHONE NUMBER 512-471-9361

Report Title

Electrostatic Control of Protein-Surface Interactions

ABSTRACT

The long-term vision of this research is to develop, characterize, and exploit surface chemistries that create stable protein-surface interactions by mimicking biological interfaces. We are pursuing this goal by chemically functionalizing well-defined and well-characterized surfaces with structured peptides. These peptides, which can be synthesized with any residual functional group, generate an electrostatic surface that will interact with proteins introduced from solution with chemically- and structurally-encoded specificity. In the current grant period, we have focused on expanding the kinds of surface reaction chemistries and secondary peptide structures under investigation and exploring possible heterogeneity on our chemically functionalized surfaces using high-resolution microscopy techniques. We are pursing a collaboration with Dr. Ron Elber investigating molecular dynamics simulations of surface-bound peptides. These developments demonstrate the impact our work will continue to make on the field of biological materials science.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
02/15/2012	3.00	Ignacio F. Gallardo, Lauren J. Webb. Demonstration of ?-Helical Structure of Peptides Tethered to Gold Surfaces Using Surface Infrared and Circular Dichroic Spectroscopies, Langmuir, (02 2012): 0. doi: 10.1021/la204927q
08/02/2013	5.00	Annette F. Raigoza, Jason W. Dugger, Lauren J. Webb. Review: Recent Advances and Current Challenges in Scanning Probe Microscopy of Biomolecular Surfaces and Interfaces, ACS Applied Materials & Interfaces, (07 2013): 0. doi: 10.1021/am4018048
08/29/2011	2.00	Ignacio F. Gallardo, Lauren J. Webb. Tethering Hydrophobic Peptides to Functionalized Self-Assembled Monolayers on Gold through Two Chemical Linkers Using the Huisgen Cycloaddition, Langmuir, (12 2010): 18959. doi: 10.1021/la1036585
11/19/2012	4.00	Annette F. Raigoza, Lauren J. Webb. Molecularly Resolved Images of Peptide-Functionalized Gold Surfaces by Scanning Tunneling Microscopy, Journal of the American Chemical Society, (11 2012): 0. doi: 10.1021/ja309632m
TOTAL:	**4**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

24. Symposium: “Electrostatics and Polarization in Biophysical Chemistry: in silico, in vitro, and in vivo,” American Chemical Society National Meeting, Indianapolis, IN, September
23. Symposium: “Physical Chemistry of Interfaces and Nanomaterials,” SPIE NanoScience and Engineering Conference, San Diego, CA, August
22. Workshop: “Protein Electrostatics,” Telluride Science Research Center, Telluride, CO, July
21. Workshop: “Chemistry and Dynamics in Complex Environments (CHEM-DiCE),” Telluride Science Research Center, Telluride, CO, June
20. Symposium: “New Frontiers and Challenges in Biomaterials Analysis,” American Chemical Society National Meeting, New Orleans, LA, April
19. University of Michigan, Department of Chemistry, Ann Arbor, MI, March
18. University of Iowa, Department of Chemistry, Iowa City, IA, March
17. Rice University, Department of Chemistry, Houston, TX, March
16. University of Pennsylvania, Department of Chemistry, Philadelphia, PA, February
15. University of Wisconsin-Madison, Department of Chemistry, Madison, WI, February
14. Baylor University, Department of Chemistry and Biochemistry, Waco, TX, February
13. University of Miami, Department of Chemistry, Coral Gables, FL, January
12. NSF CCI: Chemistry at the Space-Time Limit, University of California, Irvine, Irvine, CA, January2012
11. Indiana University, Department of Chemistry, Bloomington, IN, November
10. Brigham Young University, Department of Chemistry and Biochemistry, Provo, UT, Nov
9. University of Utah, Department of Chemistry, Salt Lake City, UT, November
8. University of California – San Diego, Department of Chemistry and Biochemistry, La Jolla, CA, October
7. Columbia University, Department of Chemistry, New York, NY, October
6. Stony Brook University, Department of Chemistry, Stony Brook, NY, October
5. City College of City University of New York, Department of Chemistry, New York, NY, October
4. Workshop: “Reactive Chemical Systems,” Army Research Office, Providence, RI, October
3. University of Washington, Department of Chemistry, Seattle, WA, September
2. Symposium: “Biotic/Abiotic Interfaces,” American Chemical Society National Meeting, Philadelphia, PA, August
1. Symposium: “Solvent Dynamics and Biomolecular Interfaces: Experiment and Theory,” American Chemical Society National Meeting, Philadelphia, PA, August

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper	
12/15/2010	1.00	Gallardo, I. F., Webb, L. J.. Tethering Hydrophobic Peptides to Functionalized Self-Assembled Monolayers on Gold through Two Chemical Linkers Using the Huisgen Cycloaddition, Langmuir (11 2010)

TOTAL: **1**

Number of Manuscripts:

Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Jason Dugger	1.00	
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Annette Raigoza	1.00
FTE Equivalent:	**1.00**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Lauren Webb	0.08	
FTE Equivalent:	**0.08**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Germain Martinez	0.00	BS
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Technology Transfer

1) Submissions and Publications of This Reporting Period

a) Papers published in peer-reviewed journals

1. Raigoza, A. F.[+]; Dugger, J. W.[+]; Webb, L. J. "Review: Recent Advances and Current Challenges in Scanning Probe Microscopy of Biomolecular Surfaces and Interfaces." *ACS Appl. Mater. Interface.* **2013**, *in press*, DOI: 10.1021/am4018048.
[+]*These authors contributed equally.*

2. Raigoza, A. F. and Webb, L. J. "Molecularly Resolved Images of Peptide-Functionalized Gold Surfaces by Scanning Tunneling Microscopy." *J. Am. Chem. Soc.* **2012**, *134*, 19354-19357.

b) Papers published in non-peer-reviewed journals

none

c) Presentations

i. Presentations at Meetings

36. Workshop: "Chemistry and Dynamics in Complex Environments (CHEM-DiCE)," Telluride Science Research Center, Telluride, CO, June 2013
35. Symposium: "New Frontiers and Challenges in Biomaterials Analysis," American Chemical Society National Meeting, New Orleans, LA, April 2013
34. University of Michigan, Department of Chemistry, Ann Arbor, MI, March 2013
33. University of Iowa, Department of Chemistry, Iowa City, IA, March 2013
32. Rice University, Department of Chemistry, Houston, TX, March 2013
31. University of Pennsylvania, Department of Chemistry, Philadelphia, PA, February 2013
30. University of Wisconsin-Madison, Department of Chemistry, Madison, WI, February 2013
29. Baylor University, Department of Chemistry and Biochemistry, Waco, TX, February 2013
28. University of Miami, Department of Chemistry, Coral Gables, FL, January 2013
27. NSF CCI: Chemistry at the Space-Time Limit, University of California, Irvine, Irvine, CA, January 2013
26. Indiana University, Department of Chemistry, Bloomington, IN, November 2012
25. Brigham Young University, Department of Chemistry and Biochemistry, Provo, UT, November 2012
24. University of Utah, Department of Chemistry, Salt Lake City, UT, November 2012
23. University of California – San Diego, Department of Chemistry and Biochemistry, La Jolla, CA, October 2012
22. Columbia University, Department of Chemistry, New York, NY, October 2012
21. Stony Brook University, Department of Chemistry, Stony Brook, NY, October 2012
20. City College of City University of New York, Department of Chemistry, New York, NY, October 2012
19. Workshop: "Reactive Chemical Systems," Army Research Office, Providence, RI, October 2012
18. University of Washington, Department of Chemistry, Seattle, WA, September 2012
17.Symposium: "Biotic/Abiotic Interfaces," American Chemical Society National Meeting, Philadelphia, PA, August 2012
16. Symposium: "Solvent Dynamics and Biomolecular Interfaces: Experiment and Theory," American Chemical Society National Meeting, Philadelphia, PA, August 2012

ii. Non-Peer-Reviewed Conference Proceedings

none

iii. Peer-Reviewed Conference Proceedings

none

d) Manuscripts

none

e) Books

none

f) Honors and Awards

none

g) Patents Disclosed

none

h) Patents Awarded

none

2) Supported Personnel Metrics of This Reporting Period

a) Graduate Students

1. Jason Dugger (100% supported, 8.3% FTE supported on this agreement)

b) Post Doctorates

1. Annette Raigoza (100% supported, 0% FTE supported on this agreement)

c) Faculty

1. Lauren Webb (75% supported, 8.3% FTE supported on this agreement)

d) Undergraduate Students

1. Germain Martinez (1% supported, 0% FTE supported on this agreement)

e) Graduating Undergraduate Metrics

none

f) Masters Degrees Awarded

none

g) Ph.D.'s Awarded

none

h) Other Research Staff

none

3) Technology Transfer

none

4) Scientific Progress and Accomplishments

A. Progress Summary The long-term vision of this research is to develop, characterize, and exploit surface chemistries that create stable protein-surface interactions by mimicking biological interfaces. We are pursuing this goal by chemically functionalizing well-defined and well-characterized surfaces with structured peptides. These peptides, which can be synthesized with any residual functional group, generate an electrostatic surface that will interact with proteins introduced from solution with chemically- and structurally-encoded specificity. In the last reporting periods we had demonstrated that we can synthesize and characterize gold surfaces functionalized with self-assembled monolayers (SAMs) that are terminated in structured α-

helices through (on average) two covalent linkages. In the current reporting period, we have focused on two principal research thrusts: 1) molecular-level structural resolution of peptide-terminated surfaces using high-resolution microscopy techniques; and 2) exploring the nucleation and controlled growth of fibril formation by altering the sequence of the surface-bound peptide. These thrusts resulted in two publications during the past year: 1) Raigoza, A. F.; Dugger, J. W.; Webb, L. J. "Review: Recent Advances and Current Challenges in Scanning Probe Microscopy of Biomolecular Surfaces and Interfaces." *ACS Appl. Mater. Interface.* **2013**, *in press*, DOI: 10.1021/am4018048; and 2) Raigoza, A. F. and Webb, L. J. "Molecularly Resolved Images of Peptide-Functionalized Gold Surfaces by Scanning Tunneling Microscopy." *J. Am. Chem. Soc.* **2012**, *134*, 19354-19357. We have also continued a productive collaboration with Dr. Ron Elber using molecular dynamics simulations to model the structure of surface-bound peptides. This research period has been both important for our long-term research goals and has produced exciting results that we believe significantly advance the goals of reproducible and functional bio/abio interfaces.

B. Research on Protein-Surface Interactions for the Current Reporting Period

(a) Molecular-Level Resolution of Peptide-Terminated Surfaces In the last reporting period, we described preliminary images showing molecular-level resolution of surface-bound peptides on the peptide-terminated surface using low-current ambient scanning tunneling microscopy (STM). We were motivated to perform these experiments in order to look for large-scale heterogeneities in our surfaces. Every surface characterization method we had published before this work began (i.e. ellipsometry, X-ray photoelectron spectroscopy, and infrared spectroscopy) collects surface-averaged information, and is not able to distinguish homogeneous *versus* heterogeneous distribution of any functional group on the surface. This is particularly problematic for the peptide-terminated surface because extensive aggregation of these peptides would make their utility quite limited. Previous atomic force microscopy (AFM) images, with resolutions above ~50 nm, did not appear to show any large-scale aggregation. Postdoctoral fellow Dr. Annette Raigoza was able to collect a number of STM images of surface-tethered α-helices under ambient conditions; representative images are shown in Figure 1. These images show gold terraces that are flat and somewhat pitted covered with Br-, N_3-, and peptide-terminated SAMs. Figures 1a and b show that the Br- and N_3-terminated surfaces are covered by a regular layer of a single thickness, as expected. The peptide-terminated surface (Figure 1c) is covered in regular elongated features approximately 2 nm x 3 nm, the size of the energy-minimized α-helix. Furthermore, the line scans across each surface, shown in Figure 1d, demonstrate that the peptide-terminated surface preserves the sharp stepped features of the gold terraces. Given the sensitivity of STM, we would expect that any amount of aggregation of the peptides would be easily seen as large featureless objects; the total absence of such debris is our first evidence that peptides are homogeneously distributed across the surface. The elongated features in Figure 1c cover an area of the surface that corresponds to approximately 0.11 peptides nm^{-2}. Based on the theoretical size of our peptide and the distance between reactive N_3 groups, a complete surface reaction will generate a surface with a peptide density of approximately 0.14 peptides nm^{-2}. These exciting results represent our first attempt to characterize the structures and orientations of peptides bound to the SAM surfaces at the molecular level, and such experiments will be a powerful new tool for our long-term research goals. We published these results in the past year: Raigoza, A. F. and Webb, L. J. "Molecularly Resolved Images of Peptide-

Functionalized Gold Surfaces by Scanning Tunneling Microscopy." *J. Am. Chem. Soc.* **2012**, *134*, 19354-19357. While writing this article, we realized that not only were these results novel and unique, but that we had solved several critical problems necessary for doing any scanning probe microscopy (SPM) of soft and deformable biological molecules. We therefore wrote a lengthy review article both to survey the current status of SPM on biomaterials and also to pose the most significant problems to further advancement of this important area of research to the community (Raigoza, A. F.; Dugger, J. W.; and Webb, L. J. "Review: Recent Advances and Current Challenges in Scanning Probe Microscopy of Biomolecular Surfaces and Interfaces." *ACS Appl. Mater. Interface.* **2013**, DOI: 10.1021/am4018048, *in press*).

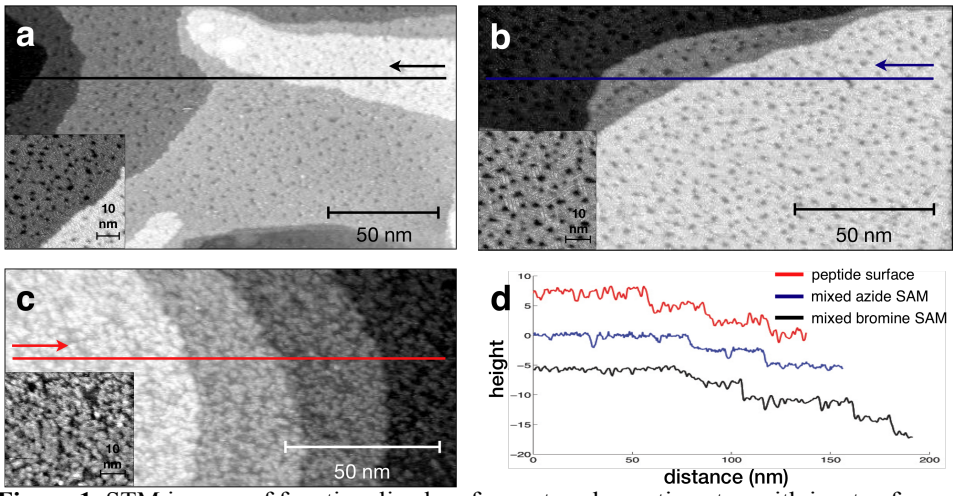

Figure 1. STM images of functionalized surfaces at each reaction step with insets of higher resolution images: (a) mixed bromine (b) mixed azide (c) peptide. Images in (a) and (b) show uniform, pitted surfaces typically formed through thiol self-assembly. The image in (c) displays features that cover the majority of the surface and are ~3 nm in size. The plot in (d) shows cross-sections of each surface, with the corresponding scan line and direction of scanning presented in the images above.

The images in Figure 1 were collected in the laboratory of Dr. Alex Kandel (University of Notre Dame, Department of Chemistry and Biochemistry), who kindly allowed us to use his home-built STM. On the strength of our ARO-supported preliminary data, we received a DURIP award to purchase such an instrument for a user facility at UT-Austin. This instrument has been purchased and we anticipate delivery and installation by the end of 2013. We believe that this new instrumentation will become a central part of the research and teaching mission of this project.

(b) Surface Functionalization With Biological Structures In the past reporting period we have begun to focus on using surface chemistry to nucleate noncovalently-assembled superstructures from the controlled aggregation of β-strand peptides into fibrils and fibers. These structures are predicted to have novel mechanical and sensing properties appropriate for a wide variety of potential biomedical applications. Fibrils are currently of significant interest to the biophysical and materials science community. While they were originally identified as the most dramatic physiological change that occurs in numerous human diseases including Alzheimer's, Parkinson's, and type II diabetes (which are associated with the aggregation of the polypeptides amyloid β, α-synuclein, and islet amyloid polypeptide (IAPP) respectively), it is now understood

that these structures are ubiquitous. Fibril structures have been formed from a large number of proteins not associated with disease states through perturbations from amino acid mutagenesis, exposure to extremes of pH, temperature, and solution concentration, or introduction of external disrupters such as detergents or high-energy radiation. These fibril structures are the thermodynamically low-energy configuration of many polypeptides regardless of amino acid sequence or function; the ability to form amyloid fibril structures under the appropriate conditions appears to be a general property of amide-based polypeptides. If fibril structure can be artificially controlled, they become an intriguing candidate for stable biocompatible building blocks for biomaterials with an enormous number of possible uses throughout medicine and materials science, such as cellular growth and culture matrices, scaffolds for artificial tissues, anti-biofouling coatings, biomarker sensors, and integrated abiological devices in biological environments, as well as numerous materials science applications.

It has been well documented through discovery-based investigations that when growing fibrils on artificial substrates and surfaces, the chemistry and structure of the substrate can alter the structure of the resulting fibril because interactions between the peptide and its chemical environment change the mechanism of the self-assembly process. Our surface peptide-functionalization method gives us complete control over all of these factors: surface charge, structure, orientation, and chemical functionality. This allows us to control and manipulate at will the size, structure, orientation, and density of surface-bound β-like peptides, and quantify the results through rigorous surface analytical techniques developed in this laboratory. With this absolute control over the structure and density of a "nucleation" site for fibril formation, combined with control over solution conditions, we propose to develop the tools to make designed amyloid fibril structures at prepared surfaces for potential use in a variety of applications mentioned above.

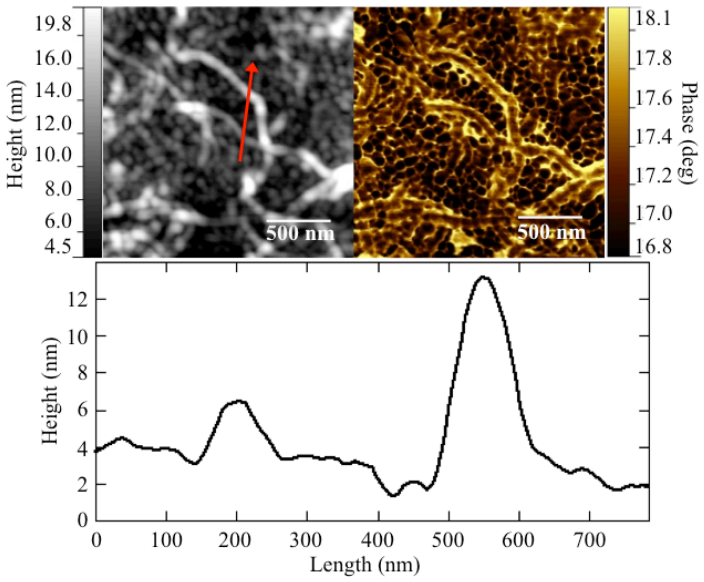

Figure 2. AFM images of a 100% N_3-terminated SAM covalently modified with β-strand peptide showing fibril structures above the SAM surface background. Top left: Topographical height; top right: phase; bottom: topographical height line scan shown by the red arrow.

We are using our well-controlled surface chemistry to build amyloid fibers of controlled size, shape, aggregation, orientation, and functionality. By using a surface-bound strand-like peptide to nucleate fibril growth, we will elucidate the rules of fibril and fiber structure at surfaces. An example of such a surface is shown in Figure 2, in which a 100% N_3-terminated SAM surface was reacted with a 100 mM solution of a β-strand peptide for 2 hrs at 70˚C. These images are dominated by two features: large areas that are indistinguishable from AFM images of the clean SAM surfaces (not shown) and long (~1 μm), thin (~100 of nm) fibril structures of varying

heights above the SAM, shown in the line scan in the bottom panel. Extensive control experiments have determined that peptides are not physisorbed to the SAM; any peptides remaining on the surface are either chemically bound to the SAM or engaged in very strong intermolecular interactions (such as in a fibril) that cannot be washed or sonicated away. These preliminary images are an exciting first step in pursuit of our goal of creating functional abiological structures from biological materials based on carefully controlled chemical inputs. Furthermore, infrared absorption spectra of the amide I ($\mathbf{A_I}$) band of the surface shown in Figure 2 reveal a multi-peak feature with absorptions near 1634, 1667, and 1694 cm^{-1}. The high-energy splitting of the $\mathbf{A_I}$ band has been consistently associated with a β-sheet structure in fibrils, rather than a β-sheet protein. The absorption at 1634 cm^{-1} suggests that these fibril structures are composed of only a few peptide strands. As the fibril grows in size, this absorption peak has been shown to decrease in energy in a manner that can be correlated to the number of strands self-assembled in the fibril. We are therefore coupling our surface-averaged FTIR experiments with high resolution microscopic imaging to determine the exact shape, structure, and size of the grown fibril structures, and determine quantitatively how these parameters change as a function of peptide sequence, solution concentration of peptide, salts, or other small molecules, time, temperature, and solution conditions such as agitation and turbidity.

Once these parameters have been explored, we will have a core capability to deliberately nucleate and build fibril structures for particular applications. For example, it has been suggested that if fibril-based materials will be useful for cellular growth matrices, they need to be highly porous in order to absorb and deliver nutrients efficiently. We propose that such materials would be formed by low-density fibril structures formed from peptide sequences that have strong intermolecular interactions (such as hydrogen bonding) with important small molecule nutrients This could be accomplished by synthesizing unnatural amino acids into the peptides to tune the function of the aggregating fibril. The successes of our proposed research will allow us to test and quantify such questions directly using known relationships between chemical inputs and surface structural outputs. We will couple our laboratory's expertise in surface chemical functionalization and unique characterization capabilities with our understanding of peptide structure, aggregation, and chemistry, to completely change the ways in which biological and abiological materials can be coaxed to interact. These successes will result in entirely new biofunctional materials.

(c) Additional SAM Functionality Biological systems regulate pH and ionic strength throughout a cell to provide a driving force for stabilizing otherwise energetically unfavorable biomacromolecular interactions. In the last reporting period, we described our initial efforts to exploit chemical complexity of our functionalized surfaces to replicate these ideas in a controlled and useful way. The motivation for this work is shown in Figure 3, which demonstrates how the noncovalent interaction of peptides with various functional groups on the surface will impact the adsorption, orientation, and subsequent reactivity of the peptides on the surface, and is therefore important to understand. For example, this figure demonstrates how examples of how a model α-helical peptide that contains positively charged lysine residues along one face and hydrophobic leucines and our reactive functional groups along the other face, might orient on these surfaces. On a methyl-terminated or amine-terminated SAM (Figure 3a, c), the helix will orient with the reactive groups oriented towards the surface because of repulsive interactions between the positively charged residues and the surface. On the other hand, the hydrophilic surface

hydroxyl-terminated and the negatively charged COOH-terminated surfaces (Figure 3b, d) will orient the peptide in the opposite direction, with the reactive groups facing away from the surface. We have very recently begun to prepare surfaces with mixed monolayers of hydroxyl, amine, and carboxylic acid functionalities with our model peptides chemically bound, not just physisorbed, to the surface in order to test these mechanisms.

We have begun to explore this problem by characterizing the interactions of model peptides with SAMs containing hydroxyl groups, amines, and carboxylic acids. Gold surfaces have been prepared with mixed thiols terminated with OH, NH_2, and COOH functionalities as well as the reactive N_3-group. Considering the pH of our reaction solution during peptide functionalization, these surfaces become hydrophilic, positively charged, and negatively charged, respectively. Model peptides (both α-helices and β-strand-like) are incubated with these surfaces, then characterized both with infrared spectroscopy and AFM.

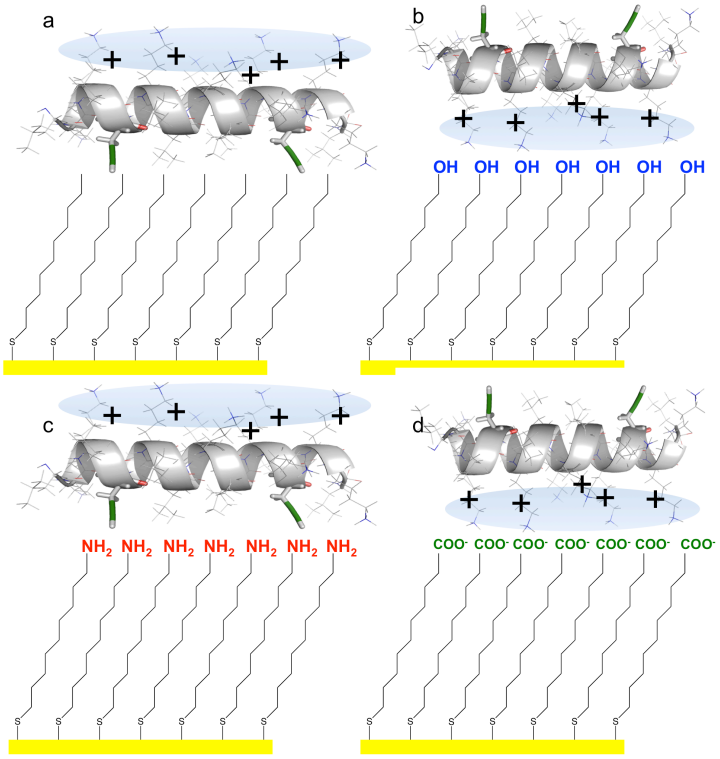

Figure 3. Cartoon showing the orientation of a α-helix identifying the positively charged lysine-containing side of the helix (blue cloud) and reactive functional groups (green sticks) on each of the functionalized surfaces: (1) methyl; (b) hydroxyl (blue); (c) amine (red); and (d) carboxylic acid (green).

Figure 4 displays grazing angle infrared spectroscopy (GRAS-IR) results of the sum of the amide I ($\mathbf{A_I}$) and amide II ($\mathbf{A_{II}}$) bands from a α-helical peptide reacted with a surface containing either 100% N_3-termination (black), or 25% N_3-terminated and 75% CH_3- (blue), OH- (red), NH_2- (purple), and COOH- (green) terminated SAM surfaces over 6 hrs of reaction time. Error bars represent one standard deviation from multiple measurements. In all cases, the amount of peptide on the surface increases (within sample error) with increasing exposure time, but the total amount of chemically bound peptide on the surface is clearly effected at

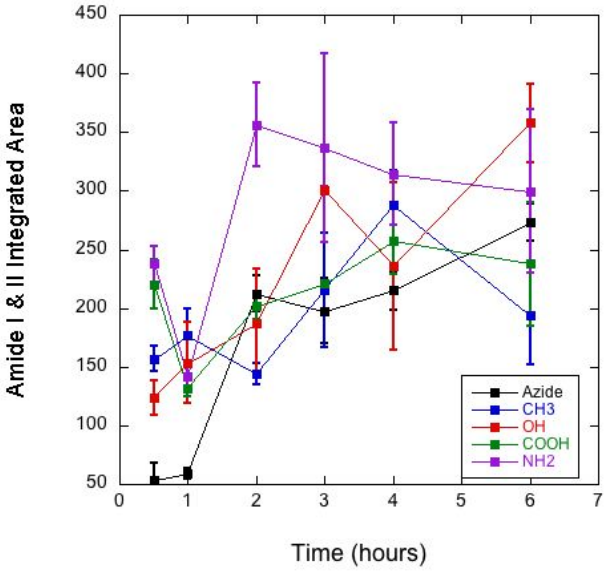

Figure 4. Sum of integrated areas of amide I and II peaks for a α-helical peptide exposed to a 100% N_3-terminated surface (black) or a 25% N_3-/75% CH_3- (blue); OH- (red); COOH- (green); and NH_2 (purple) – terminated surface for 0.5-6 hr of reaction time.

teach time point by the functionality of the monolayer. The neutral OH- and NH_2-terminated surfaces clearly show the largest amounts of bonded unstructured peptide, which at first appears somewhat surprising when compared to Figure 4. However, this may indicate the ability of the OH- group both to remain neutral under our reaction conditions is more important than any potential hydrogen bonding with the charged lysine residues. This in turn could mean that the terminal OH-groups have all hydrogen bonding needs filled by the solvent, and displacing those interactions for the peptide may be entropically unfavorable. The data in Figure 4 show differences between each surface over the reaction time, although the amount of peptide does not increase as a simple Langmuir isotherm as expected. We therefore will be spending significant time in the future establishing the kinetics of the protein-surface reaction chemistry as a function of the chemical identity of the surface.

5) Copies of Technical Reports

none